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***Research Article***

# SERO-PREVALENCE OF BOVINE LEPTOSPIROSIS IN SOUTH ANANDAMAN ISLANDS, INDIA

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**ABSTRACT: A total of 108 blood serum samples of bovine were collected from different regions of South Andaman to Regional Disease Diagnostic Laboratory (RDDL, ER, Kolkata) for sero-prevalence study of bovine leptospirosis. All the sera samples were tested by Microscopic Agglutination Test (MAT) against eight serovars of *Leptospira* spp. Out of 108 serum samples tested, 75(69.44%) were serologically positive. The most prevalent serovars were Automnalis (53.70%) followed by Sejroe (28.70%) and Hardjo (22.22%). This study suggests that bovine may have a role in maintaining Autumnalis serovar of Leptospira as reservoir in Anandaman and Nicobar Islands.**

**Key words:** Bovine Leptospirosis, MAT, Serovars, Andaman.

## INTRODUCTION

Leptospirosis, a spirochaetal zoonosis, has emerged as a serious global veterinary and public health problem. The disease has gained much importance as it is often undiagnosed (Iqbal *et al.*, 2011). Bovine leptospirosis causes financial loses to the animals from the still birth, infertility, birth of weak calves, reduced milk yield and production (Grooms 2006). The first successful isolation of leptospira in India was reported among construction workers in the village of South Andaman by Taylor and Goyle (1931). However, the first identification of leptospira was done by Adinarayanan *et al.* (1980) from Uttar Pradesh. In India, first isolation of leptospira by MAT was carried by Srivastava (1988) in man and animal from Andhra Pradesh.

Transmission of leptospirosis in human and animals occurred by the exposure to water or soil contaminated by the urine of infected animals or by direct contact with infected animals (Faine *et al.*, 1999). The genus Leptospira consists of both pathogenic and non-pathogenic species. The pathogenic leptospira is divided into more than 250 different serovars and the non-pathogenic into more than 60 serovars (Cerqueira and Picardeau 2009). Cattle

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are not only infected as accidental host but also maintenance host of specific *Leptospira* serovar strains and serves as reservoir animal.

## MATERIALS AND METHODS

### Serum samples

Serum samples of 108 cattle were sent from Senior Veterinary Officer (AH), Junglighat, Port Blair, Andaman and Nicobar Island to Regional Disease Diagnostic Laboratory (ER), Belgachia, Kolkata. The samples were collected in the months of January, 2014 and tested by Microscopic Agglutination Test (MAT) against eight serovars of *Leptospira interrogans, namely hardjo, pyrogenes, pomona, sejroe, bataviae, patoc, autumnalis,* and *australis*.

### Leptospira media and culture

Ellinghausen McCullough Johnson and Harris (EMJH) liquid media was prepared as per the standard protocol for the propagation of the leptospira culture. MAT was performed on all sera using eight reference serovars obtained from WHO National Reference Laboratory, Regional Medical Research Centre (RMRC), Port Blair,India.

### Microscopic Agglutination Test:

MAT was carried out as described by Cole *et al*. (1973) with some modification at Infectious Abortion Scheme (IAS) Laboratory, Institute of Animal Health & Veterinary Biologicals (IAH&VB), Kolkata. Leptospira live antigens required for MAT were grown in EMJH media in IAS Laboratory. For this, serovars were incubated at 30$^{o}$C for 7 days in EMJH medium. Five to seven day-old cultures at a concentration of 1-2 x10$^{8}$ organisms /ml were used as live antigen for MAT. The concentration of *Leptospira* was measured in Spectrophotometer. The sera were tested for the presence of specific antibodies against *Leptospira* serovars by MAT which was performed in 96 wells 'U' bottom "NUNC" Micro Titre plate. Firstly, all serum samples were initially diluted in three different dilutions 1:50, 1:100, 1:200 with phosphate buffer saline (PBS, pH7.4). After that, 100µl of each diluted serum was coated in to the U bottom micro titre plates, and then 100 µl of live antigen was added. The procedure was performed for all the eight serovars separately and incubated at 30$^{o}$C for 2 hours. After that, a loopful of each mixture was examined under the dark field microscope (Leica) using 20X and 40X objectives for the presence of the agglutination. One antigen control and two (positive and negative) standard serum controls were used each time. Serum sample causing 50% agglutination at 1:100 dilutions or above was taken as positive reactor as per WHO/OIE manual for leptospirosis (Guitian *et al.,* 2001, Radostites *et al.,* 2007).

## RESULTS AND DISCUSSION

Among 108 sera samples 75 were positive to any one type of serovar by MAT as shown in Table 1. That finding also clearly indicated that overall 69.44% of serum samples were positive to one or more *Leptospira* species and 33 (31.56%) were negative. All the serum samples were tested in three dilutions (1:50, 1:100 & 1:200). The positive in agglutination shown as glistering woollen ball indicated the sera are very specific to *Leptospira* serovars as depicted in Fig.1 and Fig.2. The results of this study showed that most prevalent serovars were Autumnalis, Sejroe, Hardjo and Pomona (Table 2). The less prevalent serotypes are Pattoc, Bataviae, Pyrogenes and Australis (Table 2 and Fig.3). Among the serum samples tested, 34

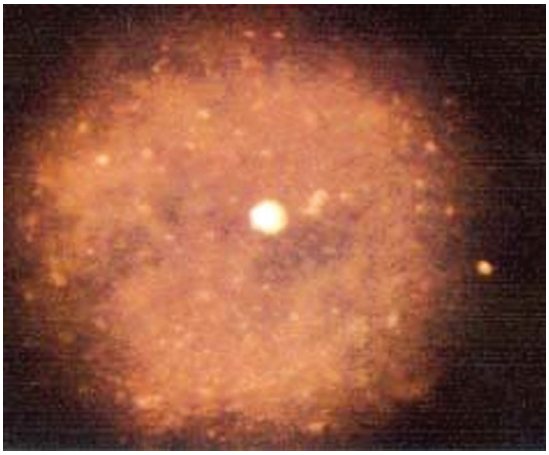

**Fig. 1: MAT at 20X under Dark Field Microscope**

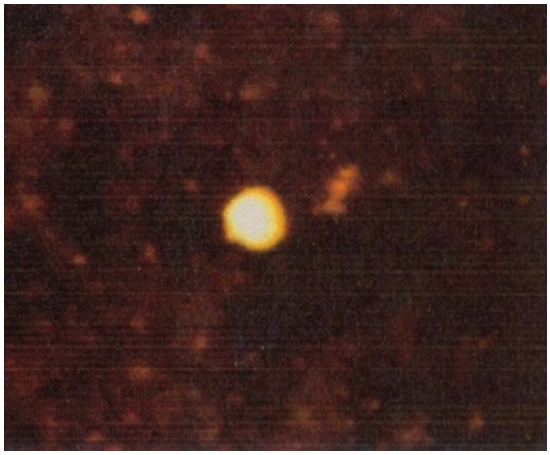

**Fig. 2: MAT at 40X under Dark Field Microscope.**

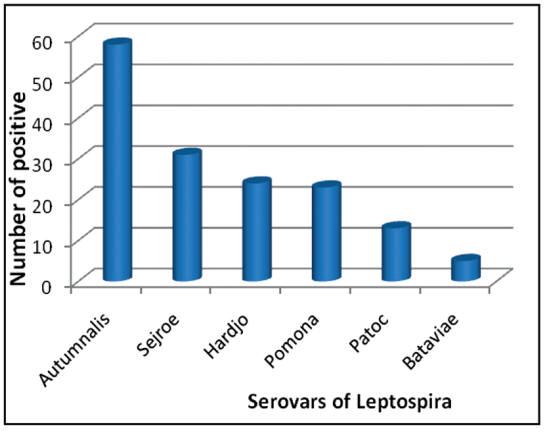


**Fig-3: Percentage of positive samples of *Leptospira* serovars.**

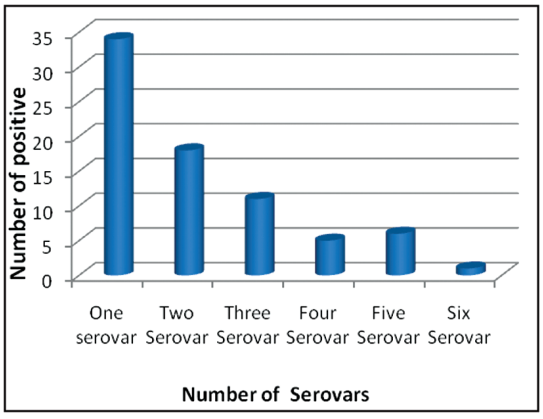


**Fig-4: Incidence of one or more serovars of positive samples.**

were positive to one serovar, 18 were positive to two serovars, 11 were positive to three serovars, 5 were positive to four serovars, 6 were positive to five serovars and 1 was positive to six serovars as shown in Table 1.These findings envisaged that most of the individual animals are infected with more than one serovar and this may cause great difficulties in controlling of the desease in that area (Fig.4).

Leptospirosis is known to occur in India since early parts of 20$^{th}$ century. The disease is common in cattle and virtually in all the states of India (Srivastava and Kumar 2003). Many leptospirosis cases in animals and humans have been reported in the rainy season and after flood and heavy rainfall. Seasonal outbreak has also been reported in coastal areas especially in the Southern pennicular region, Maharastra, 12.81% from Gujrat (Patel *et al.*, 2014), 42.50% from Odisha (Balamurgan *et al.*, 2013), 16.84%

**Table 1: Frequencies of one or more serovars of *Leptospira* sp.**

| No of serovars | Numbers of positive serovars | Frequency (%) |
|---|---|---|
| One serovar | 34 | 31.48 |
| Two serovar | 18 | 16.66 |
| Three serovar | 11 | 10.18 |
| Four serovar | 5 | 4.62 |
| Five serovar | 6 | 5.55 |
| Six serovar | 1 | 0.92 |
| **Total** | **75** | **69.44** |

from West Bengal (Mandal *et al.,* 2008). Knowledge of the prevalence of the *Leptospira* serovars is important for understanding the epidemiology of the disease and framing the public health policies aimed at prompt diagnosis and control measures. Studies of bovine leptospirosis in different parts of the world indicate that serovars responsible for reproductive losses vary depending on types of serovars that are locally endemic since leptospiral antibodies may present in the serum for a considerable period of time after infection, the sero-reactivity may indicate the present or past exposure to leptospiral antigens (Balamurgan *et al.,* 2013).

MAT is considered as the gold standard test (Wolff, 1954) or International Test (Venkataraman *et al.,* 1992) for the diagnosis of leptospirosis. It is serovar specific test and choice for sero-epidemiological studies for detecting both IgG and IgM antibodies in animal sera. The sensitivity and specificity of MAT reported in a recent study were 91.94% and 73.77%, respectively (Dassanayake *et al.,* 2009).

It is well known fact that Hardjo serovar is common in cattle (Leonard *et al.,* 2004) and present study also correlates the similar findings. In addition to this, the prevalence of serovars Autumnalis (53.70%) was highest among the all serovars in bovine species. This finding corroborates with the findings of Sarvanan *et al.* (2000) who reported that

**Table 2: Frequency distribution of *Leptospira* serovars.**

| Serovars | No. of samples reacted (1:100 dilution) | % positive samples against total samples | % frequencies against total no. of positive samples |
|---|---|---|---|
| Autumnalis | 58 | 53.70 | 77.33 |
| Sejroe | 31 | 28.70 | 41.33 |
| Hardjo | 24 | 22.22 | 32.00 |
| Pomona | 23 | 21.29 | 30.66 |
| Pattock | 13 | 12.03 | 17.33 |
| Bataviae | 5 | 4.63 | 6.66 |
| Pyrogenes | 5 | 4.63 | 6.66 |
| Australis | 2 | 1.85 | 2.66 |

majority of human disease caused by *L. interrogans* serovar Autumnalis. In another study on incidence of *Leptospira interrogans var autumnalis* in canine was highest from West Bengal by Pal *et al.*(2013). However, the highest antibody titre against the three serovars namely Autumnalis, Sejroe and Hardjo indicate that the animal population was recently affected by these three serotypes. Although, direct transmission through skin abrasions occurs, it is evident that the contaminated environment or water bodies infected by the discharge of the urine or aborted materials by either infected animals or apparently healthy carrier animals plays an important role in disease spreading. Subsequently, the animals might have been exposed to the leptospira through the reservoir animals like rodents or other infected animals shedding the leptospira in the water bodies (Balamurgan *et al.*, 2013).

## CONCLUSION

High prevalence of bovine leptospirosis in Andaman Nicobar Island indicates its significance as endemic condition. Its prevalence in apparently healthy bovine indicates the presence of the agent in the environment which may be a potential zoonotic risk to human. This study also determines the need for continuous monitoring of leptospira burden in animals and human in close proximity to each other to combat the zoonotic infection.

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